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Experimental Infection of the Laboratory Rat With the Hepatitis E Virus

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To confirm an earlier report that laboratory rats are susceptible to infection with the hepatitis E virus (HEV), we inoculated 27 Wistar rats intravenously with a suspension of a human stool known to contain infectious HEV. Stool, sera, and various tissues were collected from three rats each on days 0 (preinoculation) and 4, 7, 11, 14, 18, 21, 25, 28, and 35 postinoculation. Stool and sera specimens were examined by reverse transcription-polymerase chain reaction for the presence of HEV genomic sequences. Tissues were examined by light microscopy for detection of histopathological changes and by direct immunofluorescence for detection of HEV antigens. We detected HEV RNA in stools on day 7 in all three animals and in serum intermittently between days 4 and 35. We found HEV antigens in liver, peripheral blood mononuclear cells, spleen, mesenteric lymph nodes, and small intestine. We detected histopathology attributable to the inoculum in liver, spleen, and lymph nodes. The results confirm that HEV can replicate in laboratory rats and suggest new tissue sites for HEV replication.

KEY WORDS: HEV, RT-PCR, HEV transmission, viral hepatitis, animal model

INTRODUCTION

Hepatitis E, previously known as enterically transmitted non-A, non-B hepatitis (ET-NANBH), is the major cause of clinically overt viral hepatitis in many developing countries. The disease is noted for causing fulminant hepatitis among young adults and death among pregnant women. Hepatitis E can occur sporadically or in epidemics. Cases of hepatitis E often occur in populations virtually immune to the hepatitis A virus [Clayson et al., 1995a]. More than 50 epidemics of hepatitis E have been reported in 27 countries on the Asian, African, and North American continents over the last 30 years [for review, see Mast and Alter, 1993]. Prevention of hepatitis E is currently limited to maintaining safe drinking water; vaccines for hepatitis E are unavailable, and prophylaxis with immune serum globulin appears to have little or no protective effect [Joshi et al., 1985; Zhuang et al., 1991; Khuroo and Dar, 1992]. Research is needed to gain a better understanding of the hepatitis E virus (HEV) so that vaccines can be developed.

An animal model for hepatitis E is required for the evaluation of candidate vaccines and to learn the pathogenesis of the disease. During the last decade, experimental HEV infections have been produced by many investigators in various nonhuman primate species [for review, see Longer et al., 1993]. Most experiments have been conducted with cynomolgus macaques. Inoculation of cynomolgus macaques with HEV usually is followed by a rise in liver enzyme activity in serum between the second and the fifth weeks after inoculation. Pathological changes are usually observed in the liver when the rise in liver enzyme activity occurs. The histopathological changes are very mild compared to those observed in humans and consist of inflammatory cell infiltration and occasional hepatocyte necrosis. HEV antigens have been detected in the cytoplasm of hepatocytes and Kupffer cells before the rise in liver enzyme activity in serum and before histopathologial changes in liver. HEV particles or RNA have been detected in stool, serum, and/or bile beginning when histopathology occurs. In general, concentrations of HEV in stool

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TABLE I. Proportions of Rats Responding to Experimental Inoculation With HEV

Indicator of infection	Day postinoculation								
	4	7	11	14	18	21	25	28	35
Viral shedding[a]									
Serum	1/3	1/3	0/3	0/3	1/3	0/3	1/3	0/3	0/3
Stool	0/3	3/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
HEV Ag expression[b]									
Liver	3/3	3/3	3/3	3/3	1/3	3/3	1/3	1/3	0/3
Small intestine	0/3	0/3	1/3	0/3	2/3	0/3	1/3	2/3	2/3
Spleen	1/1	3/3	2/3	3/3	3/3	3/3	2/2	3/3	3/3
Lymph node	1/3	1/3	3/3	0/3	1/3	2/3	1/3	0/3	0/3
PBMC	2/3	2/3	1/3	0/3	3/3	1/3	2/3	3/3	0/1
Histopathological changes[c]									
Liver	0/3	3/3	3/3	1/3	1/3	1/3	2/3	1/3	2/3
Spleen	3/3	3/3	3/3	3/3	0/3	0/3	0/3	0/3	0/3
Lymph node	0/3	3/3	3/3	3/3	0/3	0/3	0/3	0/3	0/3

[a]Viral shedding was detected via RT-PCR.
[b]Viral antigen expression was detected via direct immunofluorescence.
[c]Histopathological changes were detected via light microscopy.

appear to be less than those in bile or serum. Seroconversion can usually, but not always, be detected beginning with the rise in liver enzyme activity in serum. We know little else about the pathogenesis of the disease. Sites of HEV infection, other than the liver, have not been identified. Studies in nonhuman primates have been limited by the small numbers of animals used. Experiments involving nonhuman primates typically involve small numbers of animals due to the difficulties in handling, manipulating, and housing nonhuman primates.

An alternative species that is easier to handle, manipulate, and house and that could be used in larger numbers is desired for defining the pathogenesis of hepatitis E and to evaluate candidate vaccines. Recently swine [Balayan et al., 1990; Usmanov et al., 1991; Corcoran et al., 1993], rats [Karetnyi et al., 1993], and lambs [Usmanov et al., 1994] have been shown to be susceptible to experimental infection with HEV; however, little is known about HEV infection in these species. Although the ecology of HEV remains to be determined, antibodies to HEV have been detected in wild-caught nonhuman primates [Balayan et al., 1991; Ticehurst et al., 1992], rodents [Karetnyi et al., 1993], and swine [Clayson et al., 1995b]. In addition, HEV viremia and fecal shedding have been detected in naturally infected wild swine [Clayson et al., 1995b]. Nevertheless, we know more about HEV infection in nonhuman primates than in any other species, and evaluations of candidate HEV vaccines have been conducted only in nonhuman primates [Purdy et al., 1993; Tsarev et al., 1994a].

We considered the laboratory rat as a potential model for hepatitis E. The rat is easier to raise than the nonhuman primate and is commonly used in large numbers in toxicological studies. In addition, fecal shedding and antibody responses were recently observed by investigators from the former Soviet Union in seven laboratory rats inoculated with stool containing HEV particles, suggesting that the rat is susceptible to HEV infection [Karetnyi et al., 1993]. If more was known about the pathogenesis of HEV infection in the rat, then experimental infection of the rat may become a suitable model for studying the pathogenesis of hepatitis E and for evaluating candidate vaccines.

MATERIALS AND METHODS

Animals

Weanling female Wistar rats, 250–400 g, were purchased from the National Laboratory Animal Center of Thailand and quarantined for 2 weeks. We followed guidelines of the Committee on the Care and Use of Laboratory Animals of the National Research Council during the conduct of this study. Animals were cared for in the Armed Forces Research Institute of Medical Sciences (AFRIMS) according to biosafety level 2 precautions. The study protocol was approved by the AFRIMS' Institute Animal Care and Use Committee.

Inoculum

We derived HEV isolate TK-037/92 from human feces excreted on the tenth day of disease from a 23-year-old male with acute, sporadic non-A, non-B hepatitis acquired in Nepal in 1992. The patient's illness was characterized by a peak alanine aminotransferase (ALT) of 265 IU/liter and jaundice (peak total bilirubin of 70.5 μMol/liter). Sera collected on days 7 and 9 of illness contained neither IgM to hepatitis A virus, nor IgM to hepatitis B virus core antigen, nor hepatitis B surface antigen, nor immunoglobulin to hepatitis C virus. HEV RNA was detected by reverse transcription-polymerase chain reaction (RT-PCR) in feces collected on day 10 of illness. The inoculum was a 10% (w/v) suspension of the day 10 stool in phosphate-buffered saline (PBS), pH 7.4. The suspension was clarified by centrifugations for 30 min each at 1,000 rpm and 10,000 rpm; then, the supernatant was filtered serially through 0.8 μm, 0.45 μm, and 0.2 μm sterile filters. The inoculum was stored at −70°C until injected into the

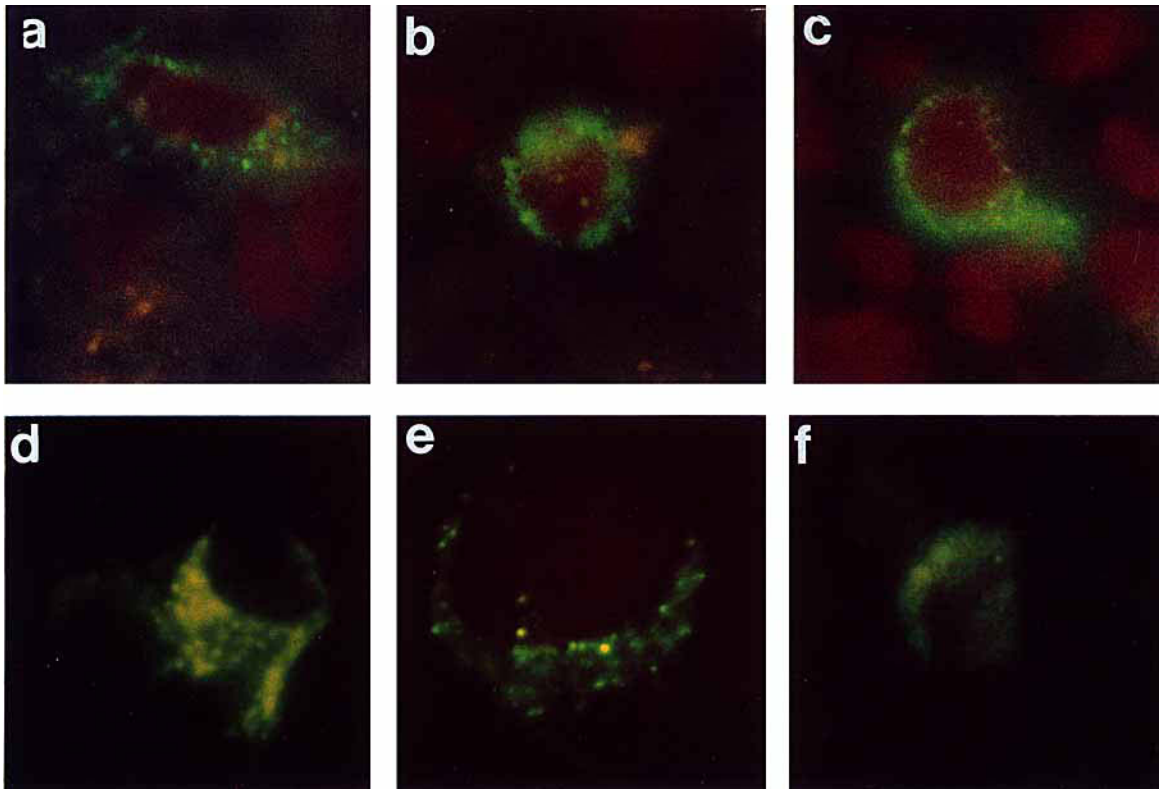


Fig. 1. HEV antigens detected via direct immunofluorescence in frozen sections of tissues from rats inoculated with HEV. **a,b:** Liver (day 4). Distinct granular fluorescence of HEV antigens distributed throughout the cytoplasms of a Kupffer cell (a) and a hepatocyte (b). **c:** Duodenum (day 18). Diffuse fluorescence of HEV antigens distributed throughout the cytoplasm of an epithelial cell. **d:** Spleen (day 21). Diffuse fluorescence of HEV antigens distributed throughout the cytoplasm of two mononuclear cells distributed in venous sinus. **e:** PBMC (day 28). Granular fluorescence of HEV antigen distributed throughout the cytoplasm of a peripheral blood mononuclear cell. **f:** Lymph node (day 21). Diffuse fluorescence of HEV antigens distributed throughout the cytoplasm of a mononuclear cell. Original magnifications ×900.

lateral tail vein of each rat with a 23-gauge hypodermic needle on day 0.

Cytopathic agents (e.g., human enteroviruses, enteric adenoviruses) were not detected in the inoculum when cultured in human rhabdomyosarcoma (RD), human embryonic kidney (HEK), human fetal lung (MRC-5), and buffalo green monkey kidney (BGM) cell lines for two passages (L. Binn, unpublished data). The virus genome titer for this inoculum was determined by RT-PCR from serial dilutions by a modification of the method of Tsarev et al. [1994b] as follows. The inoculum was diluted serially and assayed in quadruplicate for HEV RNA. The EC_{50} was determined using Probit analysis. The genomic titer was calculated to be a minimum of $10^{3.1}$ (95% CI = $10^{2.6}$–$10^{3.3}$) per 1 g of feces. Tsarev and colleagues [1994b] previously demonstrated that an inoculum containing a genomic titer of approximately 10^1 was sufficient to infect 50% of inoculated nonhuman primates.

This inoculum had been shown to contain infectious HEV by inoculation into six cynomolgus macaques lacking antibody to HEV. All six animals displayed the following indicators of infection: a rise in serum ALT activity (mean time to peak activity 31 ± 15 days), histopathology in liver biopsy specimens consistent with viral hepatitis, and HEV antigen expression in hepatocyte and Kupffer cells as directed by direct immunofluorescence. Although analysis of specimens for HEV RNA is incomplete, HEV RNA was detected in multiple specimens of bile from two of six animals (Clayson et al., unpublished data). In an earlier experiment, four swine were also injected with this inoculum. These animals displayed the following indicators of infection in the ratios indicated: viremia as detected by RT-PCR (4 of 4), viral excretion in feces as detected by RT-PCR (3 of 4), HEV antigen expression in hepatocytes and Kupffer cells as detected by direct immunofluorescence (3 of 4), and histopathology in liver biopsy specimens consistent with viral hepatitis (4 of 4) [Corcoran et al., 1993].

Specimen Collection

Three rats each were necropsied at days 0 (preinoculation) and 4, 7, 11, 14, 18, 21, 25, 28, and 35 postinoculation. Blood, stool, liver, spleen, mesenteric lymph node, stomach, duodenum, jejunum, ilium, and colon were collected from each rat at necropsy. Each blood sample was divided into two portions, one portion for

peripheral blood mononuclear cell isolation and the other for serum separation. Likewise, each tissue sample was divided into two portions, one for antigen and the other for histopathologic examination.

Histopathologic Examination

Tissues for histopathologic examination were preserved in 10% neutral buffered formalin, trimmed, embedded in paraffin, sectioned at 7 μm, and stained with hematoxylin and eosin. The tissue sections were examined under code by a veterinarian pathologist (G.D.Y.) for evidence of pathological changes.

Peripheral Blood Mononuclear Cell Isolation

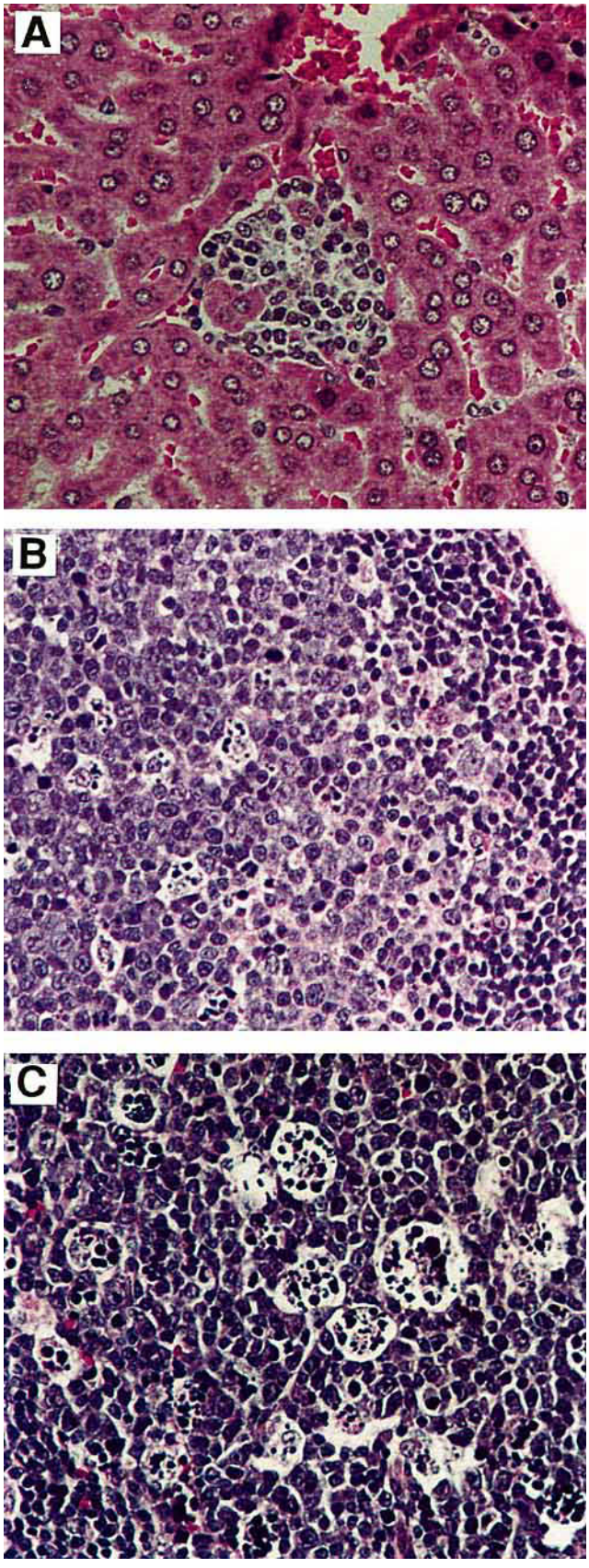
Peripheral blood mononuclear cells (PBMC) were isolated from heparinized blood by ficoll-hypaque density gradient centrifugation. Briefly, 2 ml of heparinized blood was diluted with an equal volume of Hank's balanced salt solution (HBSS; Gibco Laboratories, Grand Island, NY), overlaid on 3 ml of Histopaque-1077 (Sigma Chemical Co., St. Louis, MO), and centrifuged at 400*g* for 30 min at 20°C. Mononuclear cells were collected at the interface of blood and histopaque, washed twice with RPMI 1640 (Gibco Laboratories), and resuspended to 10^6 cells/ml in RPMI 1640 containing 10% heat-inactivated fetal calf serum (Gibco Laboratories). Aliquots of cell suspension (100 μl) were spun onto poly-L-lysine (Sigma Chemical Co., St. Louis, MO)-treated glass microscope slides by centrifugation at 800 rpm for 3 min in a cytocentrifuge (Cytospin 3; Shandon Inc., Pittsburgh, PA). The slides were air dried for 20 min and stored at −70°C until use for immunofluorescence staining.

Direct Immunofluorescence Staining

Tissues for antigen detection were snap-frozen in Tissue-Tek O.C.T. embedding medium (Miles Inc., Elkhart, IN) by immersion in dry ice-cooled petroleum ether. PBMC or 4 μm frozen tissue sections were placed on slides and examined for HEV antigens by direct immunofluorescence as described elsewhere [Krawczynski and Bradley, 1989]. The HEV-specific antibody reagent used in these studies has been thoroughly described elsewhere [Longer et al., 1993; Clayson et al., 1995b,c]. We have found the specificity of this reagent for HEV to be nearly 100% [Clayson et al., 1995c]. All specimens were tested twice under code with labeled positive and negative controls. A specimen was considered negative unless both runs of the specimen provided positive test results.

Determination of Serum Enzyme Levels

Serum was separated from clotted blood by low-speed centrifugation for 15 min at 4°C. Serum activities of ALT, aspartate aminotransferase (AST), and total bilirubin were measured by kinetic spectroscopy as described by Clayson et al. [1995c]. Determinations were made with nonhemolyzed serum held at 4°C for 24 hr or less.



Detection of HEV Antibodies

Attempts to detect antibodies to HEV in sera were made with an enzyme-linked immunosorbent assay (Diagnostic Biotechnology Co., Ltd., Singapore) using recombinant HEV fusion proteins derived from expression of strongly antigenic portions of the putative structural proteins of both HEV Burma B and Mexico 1986 isolates [Yarbough et al., 1991; Dawson et al., 1992]. The assay was performed as recommended by the manufacturer except that the anti-human IgG horseradish peroxidase conjugate was replaced with either an anti-rat IgG horseradish peroxidase conjugate (Sigma Chemical Co.) diluted 1:1,000 or an anti-rat IgM horseradish peroxidase conjugate (OEM Concepts, Toms River, NJ) diluted 1:1,000. The cutoff value for the assay was determined based on 2.5 standard deviations of the OD values of sera from 12 noninoculated rats raised in a laboratory environment.

Polymerase Chain Reaction

RNA from serum and stool was extracted with silicon dioxide as described by Clayson et al. [1995c]. Oligonucleotides, cDNA synthesis, nested DNA amplification, detection of amplified DNA, confirmation that amplified DNA contained HEV sequences, and contamination-control procedures for this study were as described by Clayson et al. [1995b]. Primers for PCR were based on sequences from the putative RNA polymerase gene region of open reading frame 1 of the Burma isolate of HEV [Tam et al., 1991]. Amplified DNA fragments were confirmed as HEV sequences by hybridization with an oligonucleotide probe specific for HEV [Clayson et al., 1995b].

RESULTS

Although none of the animals inoculated displayed any clinical signs of disease, evidence for HEV replication was found in all inoculated animals. Tissues collected at necropsy from HEV-inoculated and noninoculated animals were evaluated for the presence of viral antigens by direct immunofluorescence. HEV antigens were detected in one or more tissues from every inoculated animal but were not detected in any tissues collected from noninoculated animals (Table I). HEV antigens were detected in the cytoplasm of cells from the spleen, liver, mesenteric lymph node and duodenum and in PBMC but not in cells of the stomach, pancreas, or other areas of the digestive tract (Fig. 1). HEV antigens were detected in the livers of all animals examined between days 4 and 14 and in the livers of many animals examined through day 28 but not in any of the livers collected at day 35 (Table I). Within the liver, HEV antigens were detected in a small proportion of hepatocytes and Kupffer cells (Fig. 1a,b). The pattern of fluorescence observed in rat livers was similar to the pattern we previously observed in nearly 30 nonhuman primates and eight swine infected with HEV in our laboratory. HEV antigens were detected in the duodenum of eight animals examined on days 11, 18, 25, 28, and 35 (Table I). Within the duodenum, fluorescent cells were few, were distributed multifocally, and were found only in the epithelium (Fig. 1c). The maximal number of fluorescent duodenal cells was observed late in infection, on days 25 and 28. HEV antigens were detected in the spleens of nearly all of the inoculated animals throughout the period examined (Table I). Within the spleen, fluorescent cells were mononuclear and distributed multifocally in the venous sinus and in the lymphatic nodule area (Fig. 1d). The number of fluorescent spleen cells reached a maximum between days 18 and 21. There were a few fluorescent-stained mononuclear cells distributed in the lymphatic nodule area in mesenteric lymph nodes collected between days 4 and 25 (Table I, Fig. 1f). We also detected HEV antigens in the cytoplasm of PBMC from approximately half the inoculated animals (Fig. 1e). Within a given population of PBMC, only a few HEV antigen-containing cells were detected.

To determine whether rats respond to the HEV-containing inoculum with histopathologic signs of injury, tissues from inoculated and noninoculated animals were sectioned, stained, and examined via light microscopy. We observed changes attributable to inoculation in the liver, lymph node, and spleen but not in stomach, small intestine, and pancreas. Hepatic morphology was normal in the noninoculated animals and in all three rats examined on day 4. We observed histopathologic changes in all six animals examined between days 7 and 11, and in one or two animals in every group examined afterwards up to day 35 (Table I). The lesions observed were multifocal to diffuse accumulations of inflammatory cells next to central veins, within portal areas, and randomly within the parenchyma (Fig. 2a). The inflammatory cell infiltrates were composed almost exclusively of small lymphocytes and scattered macrophages. Occasionally, inflammatory cells encircled and isolated individual and small clumps of hepatocytes. Signs of inflammation were often associated with individual hepatocyte necrosis, which was the most marked on day 11. We observed histopathologic changes in the spleens of all animals examined between days 4 and 14 but not in any animal examined afterwards (Table I). The changes observed consisted of lymphoid hyperplasia with scattered lympholysis within germinal centers (Fig. 2B). We observed similar lympholysis in lymph node germinal centers (Fig. 2C) from all animals examined between days 7 and 14 but not in any animal examined afterwards (Table I).

Sera and stool specimens from HEV-inoculated and noninoculated rats were examined for the presence of viral RNA by RT-PCR. We detected HEV RNA in sera

Fig. 2. Histopathologic changes observed in rat tissues 11 days post-inoculation with HEV. **A:** Liver. Focal accumulation of inflammatory cells encircling and isolating hepatocytes in an area adjacent to a central vein. Inflammation is associated with hepatocyte necrosis. **B:** Spleen. Lympholysis and necrotic cellular debris within a germinal center. **C:** Lymph node. Lympholysis and necrotic cellular debris within a germinal center. Original magnification ×120.

from four inoculated rats examined on days 4, 7, 18, and 25 and in stools from three inoculated rats examined on day 7 (Table I). We did not detect HEV RNA in the stool or sera from any of the noninoculated animals.

The sera from 27 HEV-inoculated and 12 noninoculated rats were examined via kinetic spectroscopy to determine whether the inoculation resulted in a rise in ALT and total bilirubin levels in serum. We observed a significant difference in ALT levels in only two inoculated animals and in bilirubin levels of three inoculated animals. To determine whether inoculated rats produce antibodies to HEV, sera of HEV inoculated and noninoculated rats were examined via ELISA. We detected neither IgM nor IgG to HEV in any of the rats.

DISCUSSION

Our results show that HEV replicates in inoculated rats, and these findings confirm and greatly extend the findings of Karetnyi and his colleagues [1993]. We observed many of the same responses to infection as those reported for experimentally infected nonhuman primates and swine, including hepatic histopathology, viral antigen expression in liver, fecal shedding, and viremia. In nonhuman primates and swine, inflammatory cell infiltration and occasional hepatocyte necrosis are observed [Andjaparidze et al., 1986; Arankalle et al., 1988; Balayan et al., 1990; Gupta et al., 1990a,b; Usmanov et al., 1991; Ticehurst et al., 1992; Vrati et al., 1992; Corcoran et al., 1993; Longer et al., 1993]. We observed similar changes in inoculated rats, with the most frequent occurrence during the second week postinoculation. In nonhuman primates and swine, HEV antigens may be detected in the cytoplasm of hepatocytes and Kupffer cells [Krawczynski and Bradley, 1989; Ticehurst et al., 1992; Longer et al., 1993; Corcoran et al., 1993]. We observed HEV antigens in hepatocytes and Kupffer cells of inoculated rats throughout the 5 week period studied, although the most frequent occurrences were during the first 2 weeks. In nonhuman primates and swine, HEV particles or HEV RNA may be detected in bile or stool, usually when HEV antigens are observed in the liver [Balayan et al., 1983, 1990; Kane et al., 1984; Andjaparidze et al., 1986; Bradley et al., 1987; Gupta et al., 1990a,b; Uchida et al., 1990, 1991; Usmanov et al., 1991; Ticehurst et al., 1992; Vrati et al., 1992; Longer et al., 1993; Corcoran et al., 1993]. Likewise, Karetnyi and his colleagues [1993] observed HEV particles in the stool of infected rats. We detected HEV RNA in the stool from three animals early during infection. A viremia has been detected in cynomolgus monkeys [Uchida et al., 1991] and swine [Corcoran et al., 1993; Clayson et al., 1995b]. We detected HEV RNA in sera of four inoculated rats on days 4, 7, 18, and 25. The detection of HEV RNA in serum and stool shows that infection was productive in at least some animals and results in a viremia and in virus excretion.

Although HEV infection in the rat seemed similar in many ways to HEV infection in nonhuman primates, we did not observe a consistent rise in serum ALT activity as has been reported for nonhuman primates. In our study, only two rats responded to inoculation with a significant rise in serum ALT activity, although inflammation and hepatocyte necrosis consistent with viral hepatitis were frequently observed. One possibility for these results is that our test may not be sensitive for rat ALT. Another possibility is that serum levels of rat ALT may not rise after viral induced liver injury. This possibility is consistent with the observation of others that serum ALT activity is not a consistent and sensitive indicator of hepatocellular injury in rats. Histopathologic examination was far more useful than determination of serum enzyme levels for detection of liver damage in rats exposed to various hepatotoxins [Korsrud et al., 1972, 1973; Cutler, 1974; Teschke et al., 1983]. Although determination of serum ALT activity is a useful indicator of viral hepatitis in nonhuman primates, we conclude that serum ALT activity is not a useful indicator for viral hepatitis in the rat.

Unlike Karetnyi and his colleagues [1993], we were unable to detect an antibody response to HEV infection. However, our experiment ended on day 35, and Karetnyi and his colleagues could detect seroconversion only after day 57 in the seven rats they examined. Therefore, it is possible that we stopped the experiment before seroconversion occurred. It is also possible that the assay we used was not sensitive enough to detect a weak antibody response. We have previously determined that the antigens used in this assay fail to detect approximately 20% of human hepatitis E patients who have detectable viremias as determined by RT-PCR in serial serum samples [Clayson et al., 1995c].

We identified several potential sites of HEV replication in the rat that have not been previously reported for any other species. We expanded our search for sites of HEV replication to include portions of the digestive tract and lymphoid tissues. We detected histopathologic changes and/or viral antigens in the duodenum, spleen, and mesenteric lymph node and in PBMC, suggesting that HEV replication in rats occurs in these tissues along with the liver. Insofar as the duodenum, spleen, lymph node, and blood contain phagocytic cell types, and insofar as we detected HEV antigens in only a few cells in each tissue, the possibility exists that the antigen-positive cells we detected contain only phagocytized antigens from the inoculum. However, this possibility seems unlikely; cytoplasmic staining in these cells was perinuclear, suggesting viral protein replication, and we detected many antigen containing cells 35 days postinoculation, long after viral clearing should have been completed. We know of no other report that implicates or rules out these tissues as sites of HEV replication in nonhuman primates, although HEV particles were found via immune electron microscopy in a mesenteric lymph node in a single swine [Usmanov et al., 1991]. In separate studies, we have detected HEV infection in the duodenum of swine and in PBMC of swine and nonhuman primates [Corcoran et al., 1993] (Myint et al., unpublished data), indicating that HEV infection occurs at multiple sites in these animals as

well. Future studies in nonhuman primates and swine should examine the role played by nonhepatic tissues in HEV replication.

We detected HEV antigens in the liver earlier during infection than in PBMC, spleen, and lymph node, whereas we detected HEV antigens in the small intestine only late in infection. These results suggest that HEV replication occurred first in the liver and then spread to the other tissues in these animals. However, we inoculated these animals intravenously, and all tissues likely were exposed to the virus nearly simultaneously. In naturally infected animals, tissues of the digestive tract are likely to be the first tissues exposed to the virus; therefore, HEV replication in naturally infected animals may first occur in digestive tract tissues and then spread to the liver and/or other tissues. We detected HEV antigens in cells lining the small intestine, so perhaps the small intestine serves as the primary site of infection, although this remains to be determined.

Our results suggest that a rat model, when fully developed, may serve as an alternative to the nonhuman primate model for the study of HEV pathogenesis and for the evaluation of the safety and efficacy of candidate HEV vaccines. An advantage that a rat model would have over a nonhuman primate or swine model is that rats are easier to handle, manipulate, and house than nonhuman primates or swine. Another advantage is that rats may be used in greater numbers to increase statistical certainty, factors important for safety and efficacy studies. In many ways, HEV infection in the rat seemed similar to HEV infection reported in nonhuman primates and swine. However, before a rat model is fully adopted, more information is needed. For example, natural routes of exposure should be explored to optimize infectivity and to identify the primary sites of infection. A biochemical marker for HEV-induced hepatocyte injury in the rat is needed so that a simple, rapid test can be developed and used for virus infectivity experiments. Infectivity of various HEV inocula should be determined and compared in both nonhuman primates and rats. The proportions of animals with viremia and fecal shedding should be examined and compared to proportions in nonhuman primates and swine. In addition, the durations of fecal shedding and viremia have not yet been determined. Nevertheless, the advantages that a rat model would have over the nonhuman primate model warrant efforts to develop fully a rat model for evaluating candidate HEV vaccines.

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